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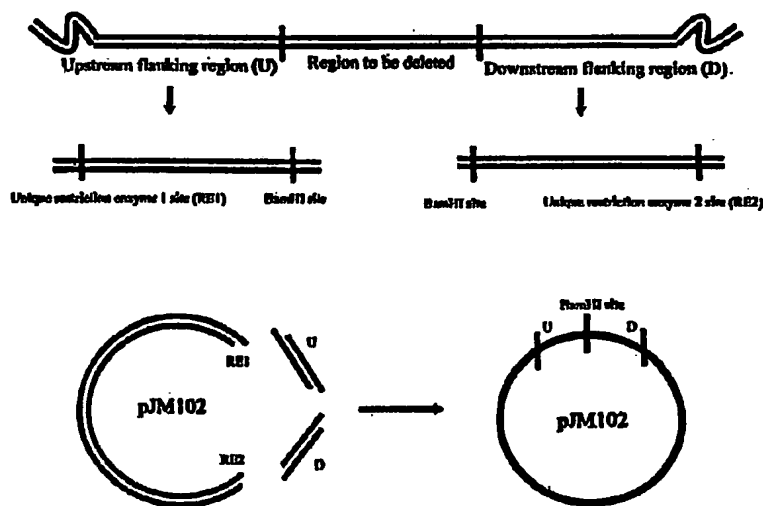
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8 March 2007

[Continued on next page]

(54) Title: PCKA MODIFICATIONS AND ENHANCED PROTEIN EXPRESSION IN BACILLUS



(57) Abstract: The present invention provides cells that have been genetically manipulated to have an altered capacity to produce expressed proteins, wherein the pckA gene has been modified or deleted. In particular, the present invention relates to Gram-positive microorganisms, such as Bacillus species having enhanced expression of a protein of interest, wherein one or more chromosomal genes have been modified and/or inactivated (e.g., pckA), and preferably wherein one or more chromosomal genes (e.g., pckA) have been modified and/or deleted from the Bacillus chromosome. In some further embodiments, one or more indigenous chromosomal regions have been modified and/or deleted from a corresponding wild-type Bacillus host chromosome.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/011821

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/32 C12N15/63 C12N9/88
ADD. C12R1/125

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12R C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, Sequence Search, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/083125 A (GENENCOR INTERNATIONAL, INC; FERRARI, EUGENIO; HARBISON, CAROLE; RASHI) 9 October 2003 (2003-10-09) the whole document, in particular p. 86, 1.1-15 and claims	1-27, 38-44, 55-64
X	US 2002/065403 A1 (EIKMANN'S BERNHARD ET AL) 30 May 2002 (2002-05-30) the whole document, in particular Example 5	1-4
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *G* document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/011821

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-27, 38-44, 55-64

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention1: Claims: 1-27,38-44,55-64

Microorganisms (in particular Bacillus) comprising a modified pckA gene, methods for enhancing production of a protein by inactivating the pckA gene and methods for obtaining Bacillus subtilis strains having enhanced protease production wherein pckA is deleted in the chromosome of said strains.

Inventions 2-28: claims: 28-37 (all partially)

Isolated nucleic acids (SEQ ID NOs:1,3,5,7,9,11,13,15,39,40,42,44,46,48,50,37,25,21,50,23,27,19,31,48,46,35,33, corresponding sequences of encoded polypeptides SEQ ID NOs:2,4,6,etc.) and host cells transformed with said nucleic acids.

Invention 29: Claims 45-54

Methods for obtaining an altered Bacillus strain expressing a protein of interest, wherein said Bacillus host cell comprises the pckA gene and has furthermore one or more chromosomal genes inactivated.

Invention 30: Claims: 65-74

Methods for enhancing the expression of a protein of interest in Bacillus, wherein said Bacillus host strain comprises a deletion of an indigenous chromosomal region.

Invention 31: Claims 75-81

Methods of enhancing the expression of a protein of interest in Bacillus wherein the nucleic acid for the protein of interest is obtained by transcriptome DNA array analysis.

Invention 32: Claims 82-85

Methods for enhancing the expression of a protein of interest in Bacillus wherein an altered Bacillus strain is used which comprises a modified gene selected from the group consisting of prpC, sigD and tdh/kbl.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/011821

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RIEDEL C ET AL: "CHARACTERIZATION OF THE PHOSPHOENOLPYRUVATE CARBOXYKINASE GENE FROM CORYNEBACTERIUM GLUTAMICUM AND SIGNIFICANCE OF THE ENZYME FOR GROWTH AND AMINO ACID PRODUCTION" JOURNAL OF MOLECULAR MICROBIOLOGY AND BIOTECHNOLOGY, HORIZON SCIENTIFIC PRESS, WYMONDHAM,, GB, vol. 3, no. 4, 2001, pages 573-583, XP001031161 ISSN: 1464-1801 the whole document, in particular Table 1 and paragraph bridging p.578/579	1-4
X	EP 0 723 011 B (AJINOMOTO CO., INC) 3 July 2002 (2002-07-03) the whole document, in particular the claims	1-3
P,X	US 2004/241831 A1 (PARK YOUNG HOON ET AL) 2 December 2004 (2004-12-02) the whole document, in particular paragraph [0012]	1-3
A	WO 03/070963 A (GENENCOR INTERNATIONAL, INC; BRON, SIERD; FERRARI, EUGENIO; TJALSMA, H) 28 August 2003 (2003-08-28)	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2005/011821

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 03083125	A	09-10-2003	AU 2003228393 A1 CA 2480277 A1 EP 1495128 A1 JP 2005521407 T	13-10-2003 09-10-2003 12-01-2005 21-07-2005
US 2002065403	A1	30-05-2002	AT 338133 T AU 6410400 A BR 0004957 A CA 2322555 A1 CN 1308125 A DE 19950409 A1 EP 1094111 A2 HU 0004115 A2 ID 27693 A JP 2001149086 A MX PA00009958 A PL 343339 A1 SK 15292000 A3 ZA 200005843 A	15-09-2006 26-04-2001 29-05-2001 20-04-2001 15-08-2001 26-04-2001 25-04-2001 28-09-2002 26-04-2001 05-06-2001 28-04-2005 23-04-2001 03-12-2001 17-05-2001
EP 0723011	B	03-07-2002	AT 220099 T AU 682547 B2 AU 8099194 A BR 9407625 A CA 2169170 A1 CN 1133615 A CZ 9600524 A3 DE 69430919 D1 DE 69430919 T2 EP 0723011 A1 HU 73690 A2 WO 9506114 A1 PL 313119 A1 SK 20496 A3 US 5876983 A US 5919694 A	15-07-2002 09-10-1997 21-03-1995 21-01-1997 02-03-1995 16-10-1996 12-06-1996 08-08-2002 27-02-2003 24-07-1996 30-09-1996 02-03-1995 10-06-1996 06-11-1996 02-03-1999 06-07-1999
US 2004241831	A1	02-12-2004	AU 2004225550 A1 BR PI0409192 A CN 1768132 A EP 1611232 A1 JP 2006521789 T WO 2004087895 A1 MX PA05010725 A	14-10-2004 11-04-2006 03-05-2006 04-01-2006 28-09-2006 14-10-2004 15-12-2005
WO 03070963	A	28-08-2003	AU 2003208922 A1 EP 1472347 A2	09-09-2003 03-11-2004